RESPONSE OF THE SKIN OF MICE TO METHYL ETHER OF VITAMIN A AND VITAMIN A PALMITATE*

ANN H. RADEMACHER, M.A. AND WILLIAM MONTAGNA, PH.D.

Interest in the compounds of vitamin A as therapeutic agents for skin disorders has stimulated a number of researches on their effects on the skin. Investigators have indicated that following the application of the unsaturated compounds of vitamin A to the skin of rats, rabbits and mice, there is a complete epilation of the area treated (7, 8, 9, 10, 11, 12, 13). These studies, however, did not take into account the stages of the hair growth cycle in the skin when the substances were applied. This paper reports the changes which occur after the application of methyl ether of vitamin A and vitamin A palmitate to the skin of mice, the hair follicles of which were in different known stages of growth.

MATERIALS AND METHODS

Thirty-two male and female C57 black mice were selected at 1½ to 2½ months of age, when their first hair growth cycle was completed, and the hair on the back was in the resting or telogen phase. Controlled growth of the follicles was obtained by plucking the club hairs (3, 5). Eight days before the application of the compounds, a small region of the lower right back was plucked. This area will be referred to as *region 1*. Four days later a second region, *region 2*, immediately anterior to region 1, was plucked. Eight days from the plucking of region 1, *region 3* was plucked in the upper right region of the back. The entire left side of the back, *region 4*, was not plucked, and the hair follicles remained quiescent or in telogen. Immediately after the plucking of region 3 the entire back was treated with the compounds as described below.

In one experiment the backs of mice were treated with ½ cc of methyl ether of vitamin A* in 95% alcohol (1.75 mg methyl ether of vitamin A/cc 95% alcohol). In a second experiment mice were treated with ½ cc of vitamin A palmitate† (20,000 units/cc 95% alcohol). All of the animals were treated with these compounds every other day for 24 days and the condition of the skin was recorded. Control mice were treated with 95% alcohol alone for 24 days. Records were kept of the color of the skin of the four regions, of the phases of hair growth, and of the gross condition of the skin.

Biopsy specimens were taken at 12 and 24 days from each of the four regions in animals from each experimental group. One-half of each tissue was fixed in Helly's fluid and one-half in trichloroacetic acid. The tissues were dehydrated, embedded in paraffin, and sectioned. Five micron sections of tissues fixed in Helly's fluid were stained with toluidin blue buffered to pH 5.0. Other sections were stained with the periodic acid-Schiff method of McManus (14); control sections were digested with saliva before they were treated with this technic. Ten micron sections of the tissues fixed in trichloroacetic acid were stained with the method of Barrnett and Seligman (2) for the demonstration of sulfhydryl and disulfide groups.

* Kindly provided by Dr. S. E. Svenson from Hoffmann-La Roche, Inc.

† Obtained from Hoffmann-La Roche, Inc., through the courtesy of the Desitin Chemical Co.

From the Department of Biology, Brown University, Providence, Rhode Island.

Received for publication June 24, 1955.

This work was supported in part by a grant from the United States Public Health Service, RG2125(C4).

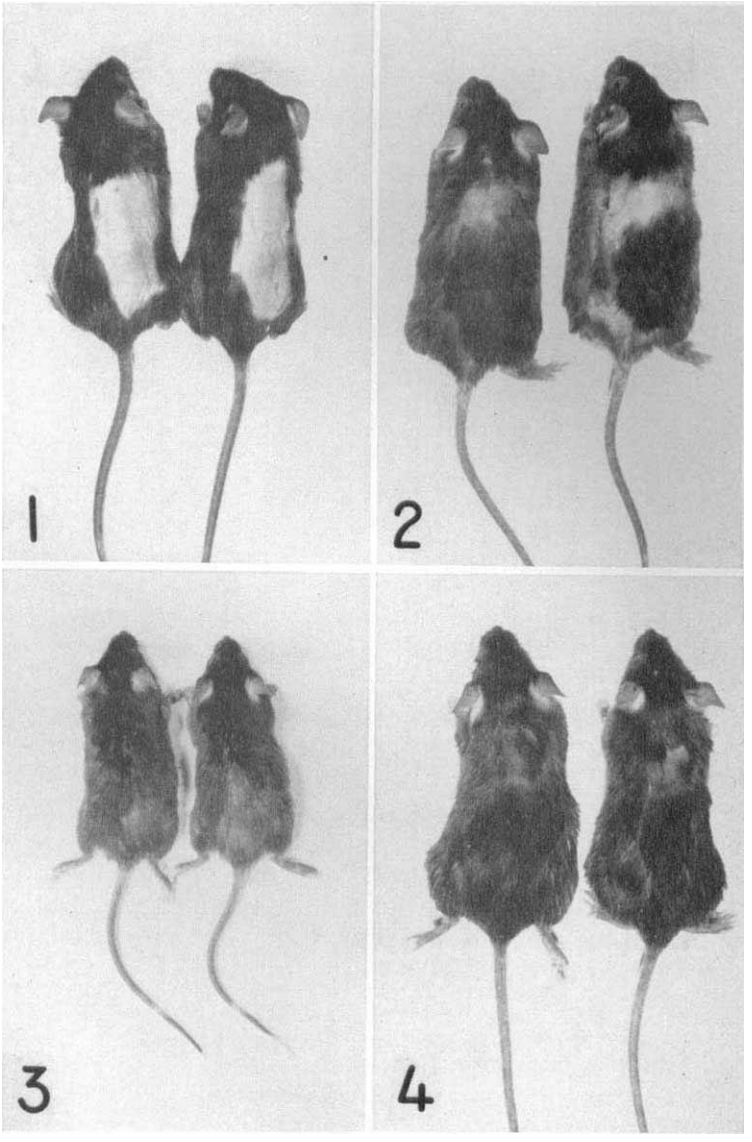

PLATE 1

FIG. 1. Two animals just before treatment with the compounds. Regions 1, 2 and 3 have been plucked at different intervals. On gross examination region 1 appeared bluish.

FIG. 2. Twelve days after the beginning of applications of methyl ether of vitamin A. Regions 1 and 2, on the posterior part of the back, have normal growing hair. Hair growth is just beginning in region 3. Hair is beginning to fall out in region 4, which is in the supra-scapular region.

FIG. 3. Twenty-four days after the beginning of treatment with methyl ether of vitamin A. Region 1 is nearly all epilated, and regions 2 and 3 are losing the hair. Region 4 has growing hair.

FIG. 4. Animals prepared as in Figure 1, treated every other day with alcohol for 12 days. Notice the regrowth of hair in regions 1, 2, and 3. Compare with Figure 2.

OBSERVATIONS

The essential points of this investigation depend on an understanding of the stages of hair growth in the mouse. Anagen, the period of active cell proliferation and development in hair follicles, is divisible into 6 recognizable substages (Chase, 1951): *anagens I* to *IV* occupy the first 7 to 8 days of the cycle and are concerned with the elaboration of the follicles and the beginning of the formation of the hair; in *anagen V*, 8 to 9 days after the initiation of growth, the tip of the newly formed hair reaches the surface of the epidermis; the hair continues to grow for the next 8 or 9 days, a period identified as *anagen VI*. After growth ceases, a club hair is formed and the follicle shrinks to a mere vestige. The transition period between anagen and the subsequent quiescent period, known as *telogen*, is called *catagen*. For some unknown reasons, when the club hairs are plucked their follicles become active practically at once. Thus, at the time of the application of the compounds used in this study, the follicles in *region 1* were in anagen V, those in *region 2* were in anagen III, those in *region 3* had just been plucked and growth had not yet begun, and *region 4* contained follicles in telogen. Grossly, the skin of these regions appeared blue-black, gray-pink, pink and pink respectively.

Region 1. The hair continued to grow for the next 11 days after the beginning of applications of methyl ether of vitamin A. This is an interval comparable to normal anagen VI and catagen. During this time the epidermis became flaky and scaly. On the 16th day after the start of the treatment the hairs began to fall out and after 22 days region 1 was denuded and pink. The surface of the epidermis became scaly and lesions appeared. On the 23rd day the area became bluish, indicating that a new growth cycle was under way. Skin specimens removed at 12 and 24 days showed that the skin was histologically normal, except for a mild hypertrophy of the epidermis. The distribution of —SH groups in the skin and hair follicles was normal (6) (Fig. 5), as was also the distribution of glycogen. In the animals treated with vitamin A palmitate the skin was similar to that described just above. The skin of the control animals treated with 95% alcohol alone showed no deviation from that of the normal untreated control animals.

Region 2. The skin in this region behaved in a similar manner when methyl ether of vitamin A and vitamin A palmitate were used. Visible growth of hair occurred 6 days after the start of application of the compounds and continued for the next 10 days. Hair began to fall out after 17 days; by 22 days the area was completely epilated, the epidermis was flaky and pink, and small lesions had developed. The skin was histologically normal 12 days after the initiation of the treatment except that the epidermis was slightly hypertrophied. The epidermis, hair follicles and sebaceous glands suffered damage at the completion of the hair growth cycle, but this was quickly repaired as soon as the new growth cycle started. By 24 days the follicles were again in early anagen of the new growth cycle (Fig. 6). The epidermis was still hypertrophied and a few hair follicles were cystic. The distribution of —SH and —S—S— groups in the skin at 12 and 24 days was normal.

Region 3. During the first week of treatment with methyl ether of vitamin A the epidermis became flaky and scaly. The entire area assumed a blue color on

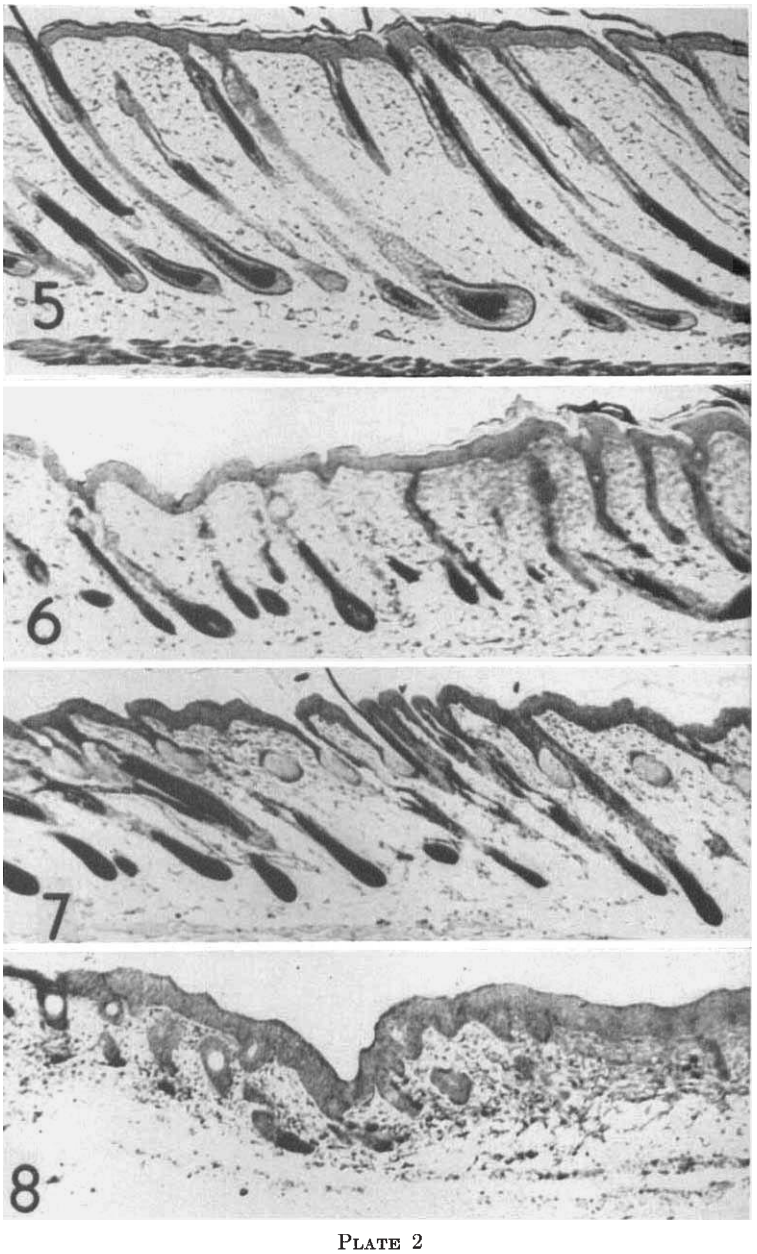

PLATE 2

FIG. 5. Skin from region 1, 12 days after the start of treatment with methyl ether of vitamin A, demonstrating the distribution of -SH groups, which is normal.

FIG. 6. Skin from region 2, 24 days after the start of treatment with methyl ether of vitamin A. The epidermis is mildly hypertrophied but a new hair generation is starting. Stained with toluidin blue.

FIG. 7. Skin from region 3, 12 days after the start of treatment. The follicles are in anagen V and VI. Stained with toluidin blue.

FIG. 8. Region 4, 12 days after the start of treatment. All of the epidermal structures show gross irritation. Compare with the figures of other regions. Stained with toluidin blue.

the 9th day. Hairs emerged on the surface on the 13th day and grew until the 21st day. Hair began to fall out on the 24th day. In the animals treated with vitamin A palmitate the results were similar to those just described but less epilation occurred. After 12 days the skin was histologically normal except that the epidermis was slightly hypertrophied. After 24 days the skin was grossly damaged; the epidermis and hair follicles were hypertrophied and the sebaceous glands fragmented. The distribution of —SH and —S—S— groups was normal after 12 days and was intensified in the hypertrophied epidermal structures.

Region 4. On the 4th day and on the 7th day of treatment with the methyl ether of vitamin A, the area became flaky, and keratinous plaques with hairs attached to them were cast off. By 11 days the area was totally denuded and grossly irritated. On the 12th day the area became blue, and new hairs emerged on the surface on the 17th day. The growth was patchy since the skin still had many lesions. Growth continued until the 24th day, at which time most of the damage had been repaired. Vitamin A palmitate had essentially the same effect but the hair emerged on the 14th day and the damage was not severe. Damage to the skin occurred again on the 24th day. Histological material removed at 12 days showed extensive hypertrophy of the epidermis; the hair follicles were reduced to solid pegs of hypertrophied cells and the sebaceous glands were fragmented. In the skin removed after 24 days the follicles were in anagen VI. In contrast, the follicles in the skin of animals treated with vitamin A palmitate were in anagen V or VI at 12 days and in telogen or in anagen II or III of the next hair generation at 24 days. The distribution of —SH was more intense in those parts of the skin which showed some damage.

DISCUSSION

Other authors have found that unsaturated compounds of vitamin A, sebum, squalene, and polymers of chloroprene and isoprene produce a reversible, local depilatory effect when applied topically to the skin of mice, rats and rabbits (7, 8, 9, 10, 11, 12, 13). When the skin becomes epilated its epidermis is markedly thickened, keratinization is said to be interfered with and the hair follicles and sebaceous glands undergo "atrophic" changes. The actions of these compounds on the skin are said to be attributable to the presence of unsaturated double bonds in the molecule, which would have a direct, local, non-specific drug effect upon the epidermal structures by interfering with sulfhydryl metabolism. In contrast with the skin of these animals, large quantities of vitamin A induce practically no disturbance to the skin of guinea pigs (15). This may be important, since unlike the hairs of the mouse, the rat and the rabbit, which are relatively in phase in any given area, those of the guinea pig grow dischronously. The few hairs of the guinea pig which fall out when vitamin A is applied topically, are all club hairs (14). From this we deduced that even in the mouse, rat and rabbit only the club hairs fall out. The present investigation, then, was designed to test this hypothesis.

The four experimental fields of hair growth on the backs of mice were each out of phase with the others. When the compounds were applied, in all animals the

hairs fell out only when the follicles were quiescent. When the follicles were active the compounds had no effect upon the rate of growth (cf. Rony et al., 17). From this it seems unlikely that these compounds of vitamin A profoundly interfere *in vivo* with cutaneous sulfhydryl metabolism and keratinization. Histochemically demonstrable sulfhydryl groups were normal in all of these experimental tissues. When the hair follicles had completed their growth cycle, the club hairs fell out, and the follicles started a new cycle of growth at once. This type of epilation does not necessarily reflect an interference with keratinization but rather a nonspecific stress response of the skin to an irritant. Similarly, skin with growing hair is relatively unaffected by one application with 20-methycholanthrene, but skin with resting follicles becomes strongly damaged (1, 4, 16). The effects of the compounds of vitamin A and of the carcinogen seem to be qualitatively the same. Whatever may be the mechanism which protects skin when hair follicles are growing, this mechanism is lacking when the follicles are quiescent.

SUMMARY

When methyl ether of vitamin A and vitamin A palmitate are applied to four regions of skin in which the stages of growth of the hair follicles have been controlled by timed plucking, the greatest amount of irritation occurs in the skin with resting hair follicles. Skin with growing follicles is relatively unaffected by these compounds. In the skin with resting follicles, which suffers a great deal of damage, after continued treatment the hair follicles become active. When this happens, the damage to the skin is repaired. These experiments show that the two compounds used do not inhibit —SH groups or keratinization in the mouse. The reaction of the skin with resting follicles to them is a generalized, non-specific stress response to an irritating agent.

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